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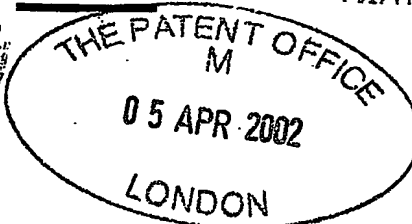
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The Patent Office

Cardiff Road
Newport
South Wales
NP9 1RH

1. Your reference

REP07020GB

2. Patent application number

(The Patent Office will fill in this part)

0207943.2

1-5 APR 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*Cambridge University Technical Services Ltd.
The Old Schools
Trinity Lane
Cambridge
CB2 1TS

8206 484 001

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

SENSORS AND THEIR PRODUCTION

5. Name of your agent *(if you have one)*

Gill Jennings & Every

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*Broadgate House
7 Eldon Street
London
EC2M 7LHPatents ADP number *(if you know it)*

745002 ✓

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country

Priority application number
*(if you know it)*Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
*(day / month / year)*8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

YES

- a) any applicant named in part 3 is not an inventor, or
 - b) there is an inventor who is not named as an applicant, or
 - c) any named applicant is a corporate body.
- See note (d).

Patents Form 1/77

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Continuation sheets of this form

Description

4 ✓

Claim(s)

1 ✓

Abstract

Drawing(s)

1 + 1 ✓

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

NO

11. For the applicant
Gill Jennings & Every

I/We request the grant of a patent on the basis of this application.

Signature

Gill Jennings & Every

Date

5 April 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Robert Edward PERRY

020 7377 1377

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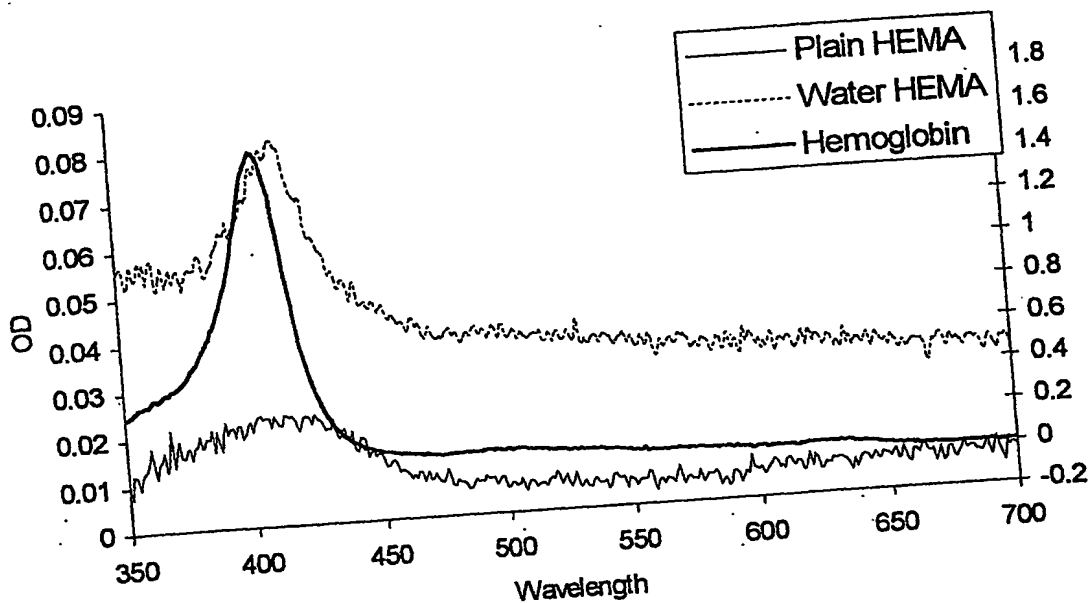
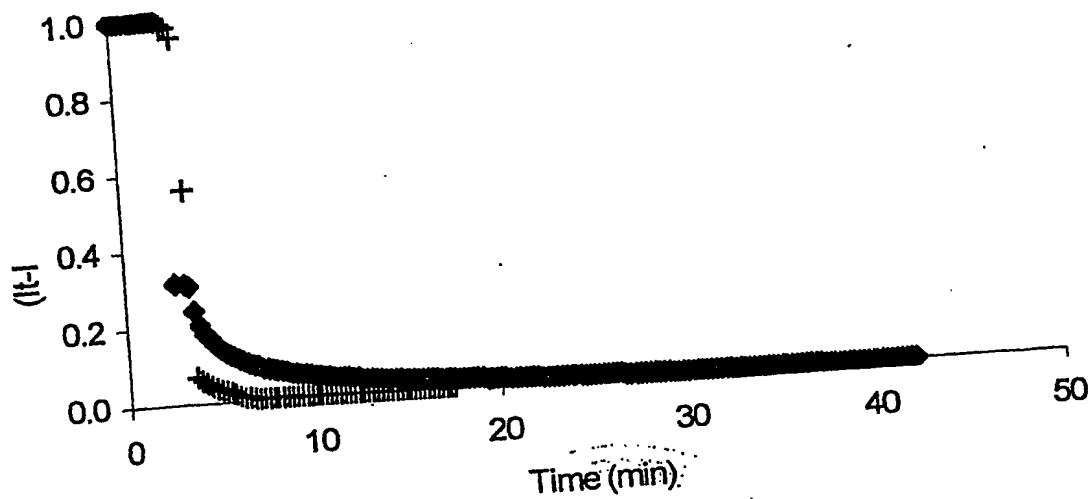


Figure 1



◆ 20 mol% EDMA, 10 mol% MAA + Microporous Sample

Figure 2

SENSORS AND THEIR PRODUCTION

Field of the Invention

This invention relates to a sensor based on a sensitive element which is a hologram.

5 Background to the Invention

WO-A-9526499 discloses a holographic sensor, based on a volume hologram. This sensor comprises an analyte-sensitive matrix having an optical transducing structure disposed throughout its volume. Because of this physical arrangement of the transducer, the optical signal generated by the sensor is very
10 sensitive to volume changes or structural rearrangements taking place in the analyte-sensitive matrix as a result of interaction or reaction with the analyte.

An alternative method of production for a holographic sensor is disclosed in WO-A-9963408. A sequential treatment technique is used, wherein the polymer film is made first and sensitive silver halide particles are added
15 subsequently. These particles are introduced by diffusing soluble salts into the polymer matrix where they react to form an insoluble light-sensitive precipitate. The holographic image is then recorded.

Summary of the Invention

An aspect of the invention is a sensor for the detection of an analyte,
20 which comprises a holographic element. The element comprises a hologram disposed throughout the volume of a support medium, wherein an optical characteristic of the hologram changes as a result of a variation of a physical property occurring throughout the volume of the medium. The medium is obtainable by formation *in situ*, preferably by the polymerisation of monomers in
25 the presence of a pore-forming agent. Though present and active in the polymerisation process, the agent is not present in the sensor and/or does not react with the analyte or the sensor. The agent may be a gas, liquid or solid; a solid may be extracted to produce pores.

The formation of additional and/or larger pores in the matrix allows greater
30 diffusion of the analyte throughout the support medium, thus making the sensor more responsive to changes in analyte concentration.

Description of the Preferred Embodiments

A holographic sensor generally comprises a holographic support medium and, disposed throughout the volume of the medium, a hologram. The support medium interacts with an analyte resulting in a variation of a physical property of the medium. This variation induces a change in an optical characteristic of the holographic element, such as its polarisability, reflectance, refractance or absorbance. If any change occurs whilst the hologram is being replayed by incident broad band, non-ionising electromagnetic radiation, then a colour or intensity change, for example, may be observed.

There are a number of basic ways to change a physical property, and thus vary an optical characteristic. The physical property that varies is preferably the size of the holographic element. This may be achieved by incorporating specific groups into the support matrix, wherein these groups undergo a conformational change upon interaction with the analyte, and cause an expansion or contraction of the support medium. A group is preferably the specific binding conjugate of an analyte species. Another method would be to change the active water content of the support medium.

A holographic sensor may be used for detection of a variety of analytes, simply by modifying the composition of the support medium. The medium preferably comprises a polymer matrix the composition of which must be optimised to obtain a high quality film, i.e. a film having a uniform matrix in which holographic fringes can be formed. The matrix is preferably formed from the copolymerisation of (meth)acrylamide and/or (meth)acrylate-derived monomers, and may be cross-linked. In particular, the monomer HEMA (hydroxyethyl methacrylate) is readily polymerisable and cross-linkable. PolyHEMA is a versatile support material since it is swellable, hydrophilic and widely biocompatible.

Other examples of holographic support media are gelatin, K-carageenan, agar, agarose, polyvinyl alcohol (PVA), sol-gels (as broadly classified), hydrogels (as broadly classified), and acrylates. Further materials are polysaccharides, proteins and proteinaceous materials, oligonucleotides, RNA, DNA, cellulose, cellulose acetate, siloxanes, polyamides, polyimides and

polyacrylamides. Gelatin is a standard matrix material for supporting photosensitive species, such as silver halide grains. Gelatin can also be photo-cross-linked by chromium III ions, between carboxyl groups on gel strands.

When the analyte is relatively large in relation to the dimensions of the pores in the polymer matrix and/or the polymer has little or no associated porosity, diffusion of the analyte into and throughout the matrix is inhibited. Thus the sensor may become slower to respond to changes in analyte concentration.

A sensor of the invention comprises a holographic support medium, which may be formed by the polymerisation of monomers or comonomers in the presence of an agent which produces a porous polymer matrix. The agent may be optimally selected for producing pores of a specific dimension. This is particularly relevant when the analyte is sterically bulky, e.g. large biological molecules such as haemoglobin.

The pore-forming agent or porogen may be a liquid, gas or solid, e.g. of particles such as bicarbonate, carbonate or PVC. When solid particles are used, they are preferably insoluble in the polymerisation mixture, such that post-polymerisation they are still present in the matrix, from which they can subsequently be removed by reaction (e.g. acid), dissolution or rinsing. When the agent is a gas, this may be bubbled through the polymerisation mixture.

An example of a pore-producing agent is water. By incorporating water into a monomer mixture, such as HEMA monomers, small pockets may be produced during the polymerisation process, resulting in a more porous polymer matrix.

For example, the agent may be a non-solvent for the polymer, which can be removed by phase separation. Another example is a salt which can be present in high concentration during polymerisation. A metal alginate could be used, and removed by washing with EDTA/acid (to take out the metal) followed by dissolution. A protein or liquid may be removed enzymatically. The agent may also be removed by physical methods, e.g. laser irradiation or ablation. A UV absorber could be heated locally, using local differences in temperature to cause pore formation.

The agent may be a gas, which could be generated *in situ*. Electrolysis or physical movement may stimulate gas formation in a suitable system. If the matrix incorporates a solvent saturated with gas, removal of the solvent will generate bubbles. Bubble formation may be stabilised by the presence of a surfactant such as Pluronic.

The following Examples illustrate the invention.

Example 1

A polymer matrix was formed by polymerisation of HEMA monomers in water and 4% methanol (w/v). For reference, a polymer was made by polymerisation of HEMA monomers in isopropanol. Upon formation, each polymer was soaked for 2 hours in 50 mg/ml haemoglobin, and the respective absorption spectra were determined, as shown in Figure 1. The absorption spectrum of a control solution of 0.25 mg/ml of haemoglobin is also shown for comparison.

The presence of water in the polymerisation mixture resulted in a more porous polymer matrix. As shown in the absorption spectra, the increased porosity of the matrix allows greater diffusion of the relatively large haemoglobin molecules, producing an absorption correlating more closely to that of the haemoglobin solution.

Example 2

A pair of holographic polymer matrices were produced, each having a monomer composition of 70% HEMA, 20% ethylene dimethacrylate (EDMA) and 10% methacrylic acid (MAA). One of the polymers was produced by polymerisation of the monomers in propanol; the other in water and 8% methanol (w/v). Holographic recording material was then disposed on each support, and the holograms recorded.

The developed holograms were immersed in an analyte sample. The response times to increasing analyte concentration are shown in Figure 2. The presence of water in the polymerisation mixture produced a microporous polymer structure, resulting in a more responsive holographic sensor.

CLAIMS

1. A sensor for the detection of an analyte, which comprises a holographic element comprising a medium and a hologram disposed throughout the volume of the medium, wherein an optical characteristic of the hologram changes as a result of a variation of a physical property occurring throughout the volume of the medium, wherein the medium is obtainable by formation *in situ* in the presence of a pore-forming agent, wherein the agent is not present in the sensor or does not react with the analyte and the sensor.
2. A sensor according to claim 1, wherein the physical property is the size of the medium.
3. A sensor according to claim 1 or claim 2, wherein the optical characteristic is the reflectance, refractance or absorbance of the holographic element.
4. A sensor according to any preceding claim, wherein the agent is a gas.
5. A sensor according to any of claims 1 to 3, wherein the agent is a liquid.
- 15 6. A sensor according to any preceding claim, wherein the agent is water.
7. A sensor according to any of claims 1 to 3, wherein the agent is a solid obtainable by extraction of the agent after the formation.
8. A sensor according to any preceding claim, wherein the medium is a polymer obtainable by the polymerisation of monomers *in situ*.
- 20 9. A sensor according to claim 8, wherein the monomers include hydroxyethyl methacrylate.